

Immunologic Nonresponsiveness to Tumors

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ABSTRACT: Over the past several years it has become clear that malignant cells express a variety of tumor associated antigens, and T cells reactive to these antigens have been identified. However, the T cells are not effective in rejecting tumors. In general, T cells that are not tolerized within the thymus have the potential to be rendered tolerant by one of three mechanisms. Immune deviation occurs when regulatory T cells which share a common precursor differentiate away from the phenotype required to effect a particular immune response. Anergy induction occurs when a T cell is stimulated through its T cell receptor in the absence of costimulation. Activation-induced cell death (AICD) is apoptosis of activated T cells upon subsequent encounter with antigen. There is emerging information that some of these mechanisms can be responsible for the lack of T cell responsiveness to tumor cells. Also, tumor cells can acquire attributes that interfere with an immune response, including down-regulation of MHC molecules or other molecules involved in antigen processing; secretion of the immunosuppressive cytokine TGF β ; and expression of the apoptosis-inducing surface molecule, Fas ligand. An expansion in our understanding of how tumor cells evade a T cell mediated death will provide insight into potential strategies to improve immunotherapeutic approaches to cancer patients.

KEY WORDS: immune tolerance, tumor immunity.

I. INTRODUCTION

There have been steady incremental improvements in cancer therapy over the years utilizing the conventional modalities of chemotherapy, radiation therapy, and surgery. With the development of alternative modalities, complementing these conventional approaches may be one way of accelerating the progress in cancer therapy. One such alternative modality is immunotherapy. There has been a recent resurgence of interest in pursuing this approach with the demonstration that these therapies have activity in cancer patients. A few specific examples include the use of systemic interleukin-2 (IL-2) in renal cell carcinoma (Burkowski, 1997) and melanoma (Philip and Flaherty, 1997); autologous tumor cell-BCG vaccines as adjuvant therapy after re-

section of colon cancer (Hoover et al., 1993); and cytokine gene-modified autologous tumor cells as vaccines in renal cell carcinoma (Simons et al., 1997) and melanoma (Abdel-Wahab et al., 1997). The problem has been that the efficacy of this approach so far has been quite poor, with very limited response rates. To improve on these early results, it is important to understand why it is that the immune system cannot reject tumor cells. It is only with this information that rational strategies for thwarting this tumor cell evasion of immune-mediated rejection can be devised. Considerable advances in the understanding of the basic mechanisms underlying immune nonresponsiveness have been made in recent years, and a limited understanding is beginning to emerge of how tumor cells specifically evade immune-mediated rejection.

II. DESPITE THE PRESENCE OF T CELLS REACTIVE TO TUMOR-SPECIFIC ANTIGENS, TUMORS ARE NOT REJECTED

Transformed cells are genetically unstable and acquire multiple mutations (Loeb, 1991; Hartwell, 1992; Lengauer et al., 1997). Some of the proteins encoded by these mutated genes should behave as antigens to which the host has not acquired tolerance through thymic mechanisms (Disis and Cheever, 1996). It has also been shown that there are a number of other nonmutated tumor-specific antigens such as the MAGE, BAGE, and GAGE gene products, and differentiation antigens such as MART-1 and gp100 (Boon and van der Bruggen, 1996). Some of these neo-antigens are presented on the cell surface through the major histocompatibility complex (MHC) class I antigen processing pathway. Cellular proteins enter this pathway after being degraded to peptides by proteasomes in the cytosol (Gaczynska et al., 1993) and are subsequently transported into the lumen of the endoplasmic reticulum by the transporter associated with antigen processing (TAP) gene products. Here they bind to MHC class I molecules and migrate to the cell surface (Cresswell et al., 1994). In this manner, intracellular mutant epitopes or other tumor-specific antigens are presented on MHC class I molecules on the cell surface that is the context in which the T-cell receptor (TCR) of CD8 positive cytolytic T lymphocytes (CTLs) recognize antigen. For example, mutations in the *ras* gene are frequently present in carcinomas (Van't Veer et al., 1989; Fearon and Vogelstein, 1990), and it has been shown that CTLs are present in humans that recognize mutant *ras*-derived peptides (Van Elsas et al., 1995; Fossum et al., 1995). Therefore, although it is clear that neo-antigens and potentially reactive T cells are present, there is the frequent failure of tumor rejection in otherwise immunocompetent individuals. Some tumor cells escape immune-mediated rejection by failing to express adequate amounts of MHC class I molecules (Garrido et al., 1997), or components of the MHC class I antigen processing pathway such as the TAP gene products (Cromme et al., 1994). However, many tumor cells express MHC class I and TAP molecules, and yet are not rejected. There-

fore, it is possible that the neo-antigen reactive T cells are rendered nonfunctional by one or more of the known mechanisms of peripheral (extra-thymic) tolerance induction, or as a result of immunological ignorance.

III. MECHANISMS OF PERIPHERAL TOLERANCE INDUCTION

Tolerance to self proteins occurs within the thymus where the processes of positive and negative selection occur. However, many self antigen-reactive T cells escape these central tolerance mechanisms in the thymus and enter the periphery (Miller and Basten, 1996). Autoimmunity is prevented by the process of peripheral tolerance induction, the three main mechanisms of which include immune deviation (Scott et al., 1994), anergy induction (Mueller et al., 1989; Rammensee et al., 1989), and activation-induced cell death (AICD) (Kabelitz et al., 1993). There have been recent reports that demonstrate that these processes may contribute to the failure of an immune-mediated rejection of tumors.

The first mechanism of peripheral tolerance induction, immune deviation, results in a functional tolerance of cell-mediated immune processes. This occurs when there is a skewing of the T-helper (TH) cell repertoire to a predominantly TH2 phenotype, diverting away from the TH1 cells that are necessary for CTL function. TH2 cells can also, in some instances, exert a direct negative influence on TH1-mediated immune responses (Cua et al., 1995). Immune deviation can take place because the T-helper cell subsets differentiate from the same precursor cells (reviewed in Abbas et al., 1996). In general, the differentiation into TH1 cells occurs when precursor cells encounter a specific peptide in the context of the appropriate MHC class II molecule and are co-stimulated with the B7 molecules in the presence of IL-12. TH1 cells then secrete cytokines, including (γ -interferon) and IL-2, which promote cellular immune responses, including the CTL-mediated rejection of tumor cells. TH2 cells differentiate from precursor cells when the latter encounter a specific peptide, are co-stimulated with the B7 molecules, and are stimulated with IL-4. TH2 cells, through the production of

cytokines such as IL-4, promote humoral immune responses. An example of immune deviation occurs during infection with the intracellular pathogen *Leishmania* (Heinzel et al., 1991). In mouse strains that have functional cell-mediated immunity that is required to clear this pathogen, TH1 cells predominate, whereas strains that fail to clear this pathogen develop a TH2 response with a functional tolerance of CTLs. Similarly, in models of T-cell-mediated autoimmunity, TH1 cells predominate, while animals manipulated in various ways to prevent autoimmunity develop a TH2 response (Falcone and Bloom, 1997). There is some evidence that this particular TH1 to TH2 immune deviation tolerance mechanism can occur in response to tumor cells that appear to be able to evade a cell-mediated immune rejection in this fashion. In several different tumor models, tumor bearing mice progressively lose TH1-cell activity and develop a relative excess of TH2 activity (Ghosh et al., 1995; Ruzek and Mathew, 1995; Maeda and Shiraishi, 1996), and in one of these models this TH1 to TH2 shift was shown to be due to TGF β (Maeda and Shiraishi, 1996). This is consistent with the recent observation that TGF β interferes with the IL-12 pathway (Pardoux et al., 1997), which is necessary for the generation of TH1 cells. Furthermore, successful rejection of a P815 tumor cell variant requires the endogenous production of IL-12 (Fallarino et al., 1996), a cytokine responsible for the differentiation of TH1 cells. Other investigators have overexpressed IL-12 in tumor cells (Zitvogel et al., 1996) or tumor-bearing animals (Tahara et al., 1994), which results in the induction of an immune response to IL-12-negative tumor cells, secondary to the production of TH1 cells (Tsong et al., 1997).

The second major mechanism of peripheral tolerance induction is through the induction of anergy. T-cell activation requires two signals (Schwartz, 1992). The first is signaling through the T-cell receptor (TCR) when it binds to peptide loaded onto an MHC molecule. The second signal comes from the binding of T-cell co-stimulatory molecules, for instance, B7-1, to their ligands (CD28) on the T-cell surface. When a T-cell receives both signals, it is activated. However, if a T-cell binds via its TCR to antigen/MHC on the target cell without sufficient co-stimulation, the T-cell is rendered anergic such that it cannot be-

come activated when restimulated with antigen (Gimmi et al., 1993). Additionally, recently it has become clear that at low B7 levels, CTLA-4 on the T-cell preferentially binds B7, which leads to the induction of anergy (Perez et al., 1997; Thompson and Allison, 1997). Another cellular interaction that is important for enhanced T-cell co-stimulation involves the binding of CD40 ligand, present on activated T cells, to CD40 on APCs that results in the up-regulation of T-cell co-stimulatory molecules on the APCs. Much like the CD28/B7 interaction, if the CD40/CD40 ligand interaction fails to occur, T-cell activation does not occur (Grewal et al., 1995). This mechanism of peripheral tolerance induction may contribute to the lack of immune responsiveness to tumors. Specifically, it has been hypothesized that one reason that T cells fail to reject tumor cells is because non-APC-derived tumor cells do not express T-cell co-stimulatory molecules (Schwartz, 1992). In fact, it has been shown that clonal anergy can be induced in T cells that are exposed to melanoma cells (Becker et al., 1993). Additional evidence supporting this hypothesis stems from the observation that overexpression of B7-1 in a variety of different tumor cells types is associated with the activation of tumor antigen-reactive T cells rejection (Chen et al., 1992; Gajewski et al., 1996; Guinan et al., 1994; Fujii et al., 1996).

The final major mechanism of peripheral tolerance induction is T-cell deletion. The administration of various antigens in a variety of different ways results in the deletion of peripheral T cells, which is preceded by T-cell activation. Examples include the administration of super antigens (Rocha and von Boehmer, 1991); exposure to the HY antigen (Zhang et al., 1992); the administration of allogeneic cells (Martin and Miller, 1989); and the intravenous administration of soluble antigens (Liblau et al., 1996). The deletion has been shown to be secondary to the induction of apoptosis and is referred to as activation-induced cell death (AICD) (Kabelitz et al., 1993). Resting T cells express very low levels of Fas, which is the surface molecule that binds to Fas ligand and transduces the signal for a cell to undergo apoptosis (Nagat and Golstein, 1995). When T cells are stimulated by antigen, there is an up-regulation of surface Fas expression and an induction of the expression of Fas

ligand (Ju et al., 1995; Dhein et al., 1995). Religation of the TCR on activated T cells then leads to apoptosis through the Fas pathway. In this manner, chronic stimulation by antigen in the periphery can result in tolerance (D'Adamio et al., 1993; Kabelitz et al., 1993). Another way that T cells can undergo apoptosis is by cytokine deprivation. Activated T cells, which have not undergone Fas-mediated apoptosis, will undergo apoptosis unless they are continuously stimulated with IL-2. IL-2 appears to prevent this apoptosis by upregulating the apoptosis inhibitory proteins Bcl-2 and Bcl-X_L (Akbar et al., 1996). Factors produced by stromal cells such as fibroblasts, epithelial cells, and endothelial cells can prevent this cytokine deprivation-induced apoptosis (Akbar et al., 1996). However, unlike T cells stimulated with IL-2, which proliferate, T cells stimulated with these stromal cell factors are quiescent (Gombert et al., 1996). These quiescent, previously activated T cells when reactivated do not undergo AICD, but rather go on to proliferate. This mechanism of peripheral tolerance induction has not been reported in tumor-specific antigen-reactive T cells in tumor-bearing animals.

IV. ACQUIRED TUMOR CELL ATTRIBUTES CAN LIMIT AN IMMUNE RESPONSE

In addition to the induction of normal tolerance mechanisms, tumor cells have been shown to acquire attributes that can have a negative impact on T-cell function. For instance, it has been shown that tumor cells can express Fas ligand (Hahne et al., 1996; O'Connell et al., 1996). Fas ligand limits normal immune responses in a physiologic fashion; however, its aberrant expression by tumor cells has the potential to delete tumor-specific CTLs resulting in an ineffectual anti-tumor immune response. Similarly, tumor cells have been shown to secrete TGF β . This cytokine functions to regulate normal immune responses. However, its ectopic production by tumor cells can have a negative impact on an anti-tumor CTL response, to the detriment of the host (Jachimczak et al., 1993).

There are additional tumor-related factors that clearly exist but are poorly understood. It has been shown in certain animal models that tumor-

specific T cells in tumor bearing animals are not tolerant, as they can be shown to be fully functional when antigen is presented in a non-neoplastic context. However, the tumor cells within an existing tumor cannot effectively elicit an immune response (Speiser et al., 1997; Wick, et al., 1997). A possible explanation that has been suggested is that the malignant cells within a tumor become surrounded by non-antigenic, normal stroma which has the effect of sequestering the tumor cells from any immune attack (Singh, et al., 1992). Others have suggested that antigen silencing occurs in tumors resulting in immunologic ignorance as an explanation for this phenomenon (Chen, 1998).

V. SUMMARY

Despite considerable advancements in the understanding of the basic mechanisms underlying immune nonresponsiveness, the specific cellular immunologic mechanisms that tumor cells exploit to avoid CTL-mediated rejection are still not very well known. Clearly, this process is complex with a variety of different peripheral tolerance mechanisms coming into play. In addition, it appears that even if tolerance is not induced, the anti-tumor CTL response is not easily sustained. Furthermore, the environment of an established tumor may be such that non-tolerant CTLs cannot mount an immune-mediated rejection within an existing tumor. It remains important to continue to study these processes, as the more that is known about how tumor cells evade rejection, the more rationally immunotherapeutic strategies can be devised.

REFERENCES

- Abbas, A. K., K. M. Murphy, and A. Sher: Functional diversity of helper T lymphocytes. *Nature*. 383:787-793 (1996).
- Abdel-Wahab, Z., C. Weltz, D. Hester, N. Pickett, C. Vervaert, J. R. Barber, D. Jolly, and H. F. Seigler: A phase I clinical trial of immunotherapy with interferon- γ gene-modified autologous melanoma cells: monitoring the humoral immune response. *Cancer*. 80:401-412 (1997).
- Akbar, A. N., N. J. Borthwick, R. G. Wickremasinghe, P. Panayiotidis, D. Pilling, M. Bofill, S. Krajewski, J. C. Reed, and M. Salmon: Interleukin-2 receptor common gamma-chain signaling cytokines regulate activated T-

- cell apoptosis in response to growth factor withdrawal: selective induction of anti-apoptotic (bcl-2, bcl-xL) but not pro-apoptotic (bax, bcl-xS) gene expression. *Eur. J. Immunol.*, 26:294-299 (1996).
- Becker, J. C., T. Brabletz, C. Czerny, C. Termeer, and E. B. Brocker: Tumor escape mechanisms from immunosurveillance: induction of unresponsiveness in a specific MHC-restricted CD4+ human T-cell clone by the autologous MHC class II+ melanoma. *Int. Immunol.*, 5:1501-1508 (1993).
- Boon, T. and P. van der Bruggen: Human tumor antigens recognized by T lymphocytes. *J. Exp. Med.*, 183:725-729 (1996).
- Bukowski, R. M.: Natural history and therapy of metastatic renal cell carcinoma: the role of interleukin-2. *Cancer*, 80:1198-1220. (1997)
- Chen, L., S. Ashe, W. A. Brady, I. Hellstrom, K. E. Hellstrom, J. A. Ledbetter, P. McGowan, and P. S. Linsley: Costimulation of anti-tumor immunity by the B7 counterreceptor for the T lymphocyte molecules CD28 and CTLA-4. *Cell*, 71:1093-1102 (1992).
- Chen, L.: Immunological ignorance of silent antigens as an explanation of tumor evasion. *Immunol. Today*, 19:27-30 (1998).
- Cresswell, P., M. J. Androlewicz, and B. Ortman: Assembly and transport of class I MHC-peptide complexes. *Ciba Found. Symp.*, 187:150-162 (1994).
- Cromme, F. V., J. Airey, M. T. Heemels, H. L. Ploegh, P. J. Keating, P. L. Stern, C. J. Meijer, and J. M. Walboomers: Loss of transporter protein, encoded by the TAP-1 gene, is highly correlated with loss of HLA expression in cervical carcinomas. *J. Exp. Med.*, 179:335-340 (1994).
- Cua, D. J., D. R. Hinton, and S. A. Stohlman: Self-antigen-induced Th2 responses in experimental allergic encephalomyelitis (EAE)-resistant mice. Th2-mediated suppression of autoimmune disease. *J. Immunol.*, 155:4052-4059 (1995).
- Dhein, J., H. Walczak, C. Baumler, K. Debatin, and P. H. Krammer: Autocrine T-cell suicide mediated by APO-1(Fas/CD95). *Nature*, 373:438-441 (1995).
- Disis, M. L. and M. A. Cheever: Oncogenic proteins as tumor antigens. *Curr. Opin. Immunol.*, 8:637-642 (1996).
- D'Adamio, L., K. M. Awad, and E. L. Reinherz: Thymic and peripheral apoptosis of antigen-specific T cells might cooperate in establishing self tolerance. *Eur. J. Immunol.*, 23:747-753 (1993).
- Falcone, M. and B. R. Bloom: A T helper cell 2 (Th2) immune response against non-self antigens modifies the cytokine profile of autoimmune T cells and protects against experimental allergic encephalomyelitis. *J. Exp. Med.*, 185:901-907 (1997).
- Fallarino, F., C. Uyttenhove, T. Boon, and T. F. Gajewski: Endogenous IL-12 is necessary for rejection of P815 tumor variants *in vivo*. *J. Immunol.*, 156:1095-1100 (1996).
- Fearon, E. R. and B. Vogelstein: A genetic model for colorectal tumorigenesis. *Cell*, 61:759-767 (1990).
- Fossum, B., A. C. Olsen, E. Thorsby, and G. Gaudernack: CD8+ T cells from a patient with colon carcinoma specific for a mutant p21-ras-derived peptide are cytotoxic towards a carcinoma cell line harbouring the same mutation. *Cancer Immunol. Immunother.*, 40:165-172 (1995).
- Fujii, H., M. Inobe, F. Kimura, J. Murata, M. Murakami, Y. Onishi, I. Azuma, T. Uede, and I. Saiki: Vaccination of tumor cells transfected with the B7-1 (CD80) gene induces the anti-metastatic effect and tumor immunity in mice. *Int. J. Cancer*, 66:219-224 (1996).
- Gaczynska, M., K. L. Rock, and A. L. Goldberg: Role of proteasomes in antigen presentation. *Enzyme Protein*, 47:354-369 (1993).
- Gajewski, T. F., F. Fallarino, C. Uyttenhove, and T. Boon: Tumor rejection requires a CTLA-4 ligand provided by the host or expressed on the tumor: superiority of B7-1 over B7-2 for active tumor immunization. *J. Immunol.*, 156:2909-2917 (1996).
- Garrido, F., F. Ruiz-Cabello, T. Cabrera, J. J. Perez-Villar, M. Lopez-Botet, M. Duggan-Keen, and P. L. Stern: Implications for immunosurveillance of altered HLA class I phenotypes in human tumors. *Immunol. Today*, 18:89-95 (1997).
- Ghosh, P., K. L. Komschlies, M. Cippitelli, D. L. Longo, J. Subleski, J. Ye, A. Sica, H. A. Young, R. H. Wiltout, and A. C. Ochoa: Gradual loss of T-helper 1 populations in spleen of mice during progressive tumor growth. *J. Natl. Cancer Inst.*, 87:1478-1483 (1995).
- Gimmi, C. D., G. J. Freeman, J. G. Gribben, G. Gray, and L. M. Nadler: Human T-cell clonal anergy is induced by antigen presentation in the absence of B7 costimulation. *Proc. Natl. Acad. Sci. U.S.A.*, 90:6586-6590 (1993).
- Gombert, W., N. J. Borthwick, D. L. Wallace, H. Hyde, M. Bofill, D. Pilling, P. C. Beverley, G. Janossy, M. Salmon, and A. N. Akbar: Fibroblasts prevent apoptosis of IL-2-deprived T cells without inducing proliferation: a selective effect on Bcl-XL expression. *Immunology*, 89:397-404 (1996).
- Grewal, I. S., J. Xu, and R. A. Flavell: Impairment of antigen-specific T-cell priming in mice lacking CD40 ligand. *Nature*, 378:617-620 (1995).
- Guinan, E. C., J. G. Gribben, V. A. Boussiotis, G. J. Freeman, and L. M. Nadler: Pivotal role of the B7:CD28 pathway in transplantation tolerance and tumor immunity. *Blood*, 84:3261-3282 (1994).
- Hahne, M., D. Rimoldi, M. Schroter, P. Romero, M. Schreier, L. E. French, P. Schneider, T. Bornand, A. Fontana, D. Lienard, J. Cerottini, and J. Tschopp: Melanoma cell expression of Fas (AP01/CD95) ligand: implications for tumor immune escape. *Science*, 274:1363-1366 (1996).
- Hartwell, L.: Defects in a cell cycle checkpoint may be responsible for the genomic instability of cancer cells. *Cell*, 71:543-546 (1992).
- Heinzel, F. P., M. D. Sadick, S. S. Mutha, and R. M. Locksley: Production of interferon- γ , interleukin 2, interleukin 4, and interleukin 10 by CD4+ lymphocytes *in vivo* during healing and progressive murine leishmaniasis. *Proc. Natl. Acad. Sci. U.S.A.*, 88:7011-7015 (1991).
- Hoover H. C., Jr., J. S. Brandhorst, L. C. Peters, M. G. Surdyke, Y. Takeshita, J. Madariaga, L. R. Muenz, and

- M. G. Hanna, Jr.: Adjuvant active specific immunotherapy for human colorectal cancer: 6.5-year median follow-up of a phase III prospectively randomized trial. *J. Clin. Oncol.*, 11:390-399 (1993).
- Jachimczak, P., U. Bogdahn, J. Schneider, C. Behl, J. Meixensberger, R. Apfel, R. Dorries, K. H. Schlingensiepen, and W. Brysch: The effect of transforming growth factor β 2-specific phosphorothioate-antisense oligodeoxynucleotides in reversing cellular immunosuppression in malignant glioma. *J. Neurosurg.*, 78:944-951 (1993).
- Ju, S., D. J. Panka, H. Cui, R. Ettinger, M. el-Khatib, D. H. Sherr, B. Z. Stanger, and A. Marshak-Rothstein: Fas (CD95)/FasL interactions required for programmed cell death after T-cell activation. *Nature*, 373:444-448 (1995).
- Kabelitz, D., T. Pohl, and K. Pechhold: Activation-induced cell death (apoptosis) of mature peripheral T lymphocytes. *Immunol. Today*, 14:338-339 (1993).
- Lengauer, C., K. W. Kinzler, and B. Vogelstein: Genetic instability in colorectal cancers. *Nature*, 386:623-627 (1997).
- Liblau, R. S., R. Tisch, S. Y. Shokat, X. Yang, N. Dumont, C. C. Goodnow, and H. O. McDevitt: Intravenous injection of soluble antigen induces thymic and peripheral T-cell apoptosis. *Proc. Natl. Acad. Sci. U.S.A.*, 93:3031-3036 (1996).
- Loeb, L.: Mutator phenotype may be required for multistage carcinogenesis. *Cancer Res.*, 51:3075-3079 (1991).
- Maeda, H. and A. Shiraishi: TGF- β contributes to the shift toward Th2-type responses through direct and IL-10-mediated pathways in tumor-bearing mice. *J. Immunol.*, 156:73-78 (1996).
- Martin, D. R. and R. G. Miller: *In vivo* administration of histoincompatible lymphocytes leads to rapid functional deletion of cytotoxic T lymphocyte precursors. *J. Exp. Med.*, 70:679-690 (1989).
- Miller, J. F. and A. Basten: Mechanisms of tolerance to self. *Curr. Opin. Immunol.*, 8:815-821 (1996).
- Mueller, D. L., M. K. Jenkins, and R. H. Schwartz: Clonal expansion versus functional clonal inactivation: a costimulatory signaling pathway determines the outcome of T-cell antigen receptor occupancy. *Annu. Rev. Immunol.*, 7:445-480 (1989).
- Nagata, S. and P. Golstein: The Fas death factor. *Science*, 267:1449-1456 (1995).
- O'Connell, J., G. C. O'Sullivan, J. K. Collins, and F. Shanahan: The Fas counterattack: Fas-mediated T-cell killing by colon cancer cells expressing Fas ligand. *J. Exp. Med.*, 184:1075-1082 (1996).
- Pardoux, C., C. Asselin-Paturel, J. Chehimi, F. Gay, F. Mami-Chouaib, and S. Chouaib: Functional interaction between TGF β and IL-12 in human primary allogeneic cytotoxicity and proliferative response. *J. Immunol.*, 158:136-143 (1997).
- Perez, V. L., L. van Parijs, A. Biuckians, X. X. Zeng, T. B. Strom, and A. K. Abbas: Induction of peripheral T-cell tolerance *in vivo* requires CTLA-4 engagement. *Immunity*, 6:411-417 (1997).
- Philip, P. A. and L. Flaherty: Treatment of malignant melanoma with interleukin-2. *Semin. Oncol.*, 24:S32-S38 (1997).
- Rammensee, H. G., R. Kroschewski, and B. Frangoulis: Clonal anergy induced in mature V β 6+ T lymphocytes on immunizing Mls-1^b mice with Mls-1^a-expressing cells. *Nature*, 339:541-544 (1989).
- Rocha, B. and H. von Boehmer: Peripheral selection of the T-cell repertoire. *Science*, 251:1225-1228 (1991).
- Ruzek, M. C. and A. Mathur: Specific decrease of Th1-like activity in mice with plasma cell tumors. *Int. Immunol.*, 7:1029-1035 (1995).
- Schwartz, R. H.: Costimulation of T lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. *Cell*, 71:1065-1068 (1992).
- Scott, B., R. Liblau, S. Degermann, L. A. Marconi, L. Ogata, A. J. Caton, H. O. McDevitt, and D. Lo: A role for non-MHC genetic polymorphism in susceptibility to spontaneous auto-immunity. *Immunity*, 1:73-83 (1994).
- Simons, J. W., E. M. Jaffee, C. E. Weber, H. I. Levitsky, W. G. Nelson, M. A. Carducci, A. J. Lazenby, L. K. Cohen, C. C. Finn, S. M. Clift, K. M. Hauda, L. A. Beck, K. M. Leiferman, A. H. Owens, S. Piantadosi, G. Dranoff, R. C. Mulligan, D. M. Pardoll, and F. F. Marshall: Bioactivity of autologous irradiated renal cell carcinoma vaccines generated by *ex vivo* granulocyte-macrophage colony-stimulating factor gene transfer. *Cancer Res.*, 57:1537-1546 (1997).
- Singh, S., S. R. Ross, M. Accena, D. A. Rowley, and H. Schreiber: Stroma is critical for preventing or permitting immunological destruction of antigenic cancer cells. *J. Exp. Med.*, 175:139-146 (1992).
- Speiser, D. E., R. Miranda, A. Zakarian, M. F. Bachmann, K. McKall-Faenza, B. Odermatt, D. Hanahan, R. M. Zinkernagel, and P. S. Ohashi: Self antigens expressed by solid tumors do not efficiently stimulate naïve or activated T cells: implications for immunotherapy. *J. Exp. Med.*, 186:645-653 (1997).
- Tahara, H., H. J. Zeh, W. J. Storkus, I. Pappo, S. C. Watkins, U. Gubler, S. F. Wolf, P. D. Robbins, and M. T. Lotze: Fibroblasts genetically engineered to secrete IL-12 can suppress tumor growth and induce antitumor immunity to a murine melanoma *in vivo*. *Cancer Res.*, 54:182-189 (1994).
- Thompson, C. B. and J. P. Allison: The emerging role of CTLA-4 as an immune attenuator. *Immunity*, 7:445-450 (1997).
- Tsung, K., J. B. Meko, G. R. Peplinski, Y. L. Tsung, and J. A. Norton: IL-12 induces T helper 1-directed anti-tumor response. *J. Immunol.*, 158:3359-3365 (1997).
- Van Elsas, A., H. W. Nijman, C. E. Van der Minne, J. Mourer, W. M. Kast, C. J. Melief, and P. I. Schrier: Induction and characterization of cytotoxic T-lymphocytes recognizing a mutated p21 ras peptide presented by HLA-A0201. *Int. J. Cancer*, 61:389-396 (1995).
- van't Veer, L. J., B. M. Burgering, R. Versteeg, A. J. Boot, D. J. Ruiter, S. Osanto, P. I. Schrier, and J. L. Bos: N-ras mutations in human cutaneous melanoma from sun-exposed body sites. *Mol. Cell. Biol.*, 9:3144-3116 (1989).
- Wick, M., P. Dubey, H. Koeppen, C. T. Siegel, P. E. Fields, L. Chen, J. A. Bluestone, and H. Schreiber: Antigenic cancer cells grow progressively in immune hosts with-

out evidence for T cell exhaustion or systemic anergy. *J. Exp. Med.*, 186:229-238 (1997).

Zhang, L., D. R. Martin, W. P. Fung-Leung, H. S. Teh, and R. G. Miller: Peripheral deletion of mature CD8+ antigen-specific T cells after *in vivo* exposure to male antigen. *J. Immunol.*, 148:3740-3745 (1992).

Zitvogel, L., P. D. Robbins, W. J. Storkus, M. R. Clarke, M. J. Maeurer, R. L. Campbell, C. G. Davis, H. Tahara, R. D. Schreiber, and M. T. Lotze: IL-12 and B7-1 costimulation cooperate in the induction of effective antitumor immunity and therapy of established tumors. *Eur. J. Immunol.*, 26:1335-1341 (1996).

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- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
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